Autonomous real-time monitoring of the abundance and activity of microorganisms in coastal waters: the Environmental Sample Processor (ESP)

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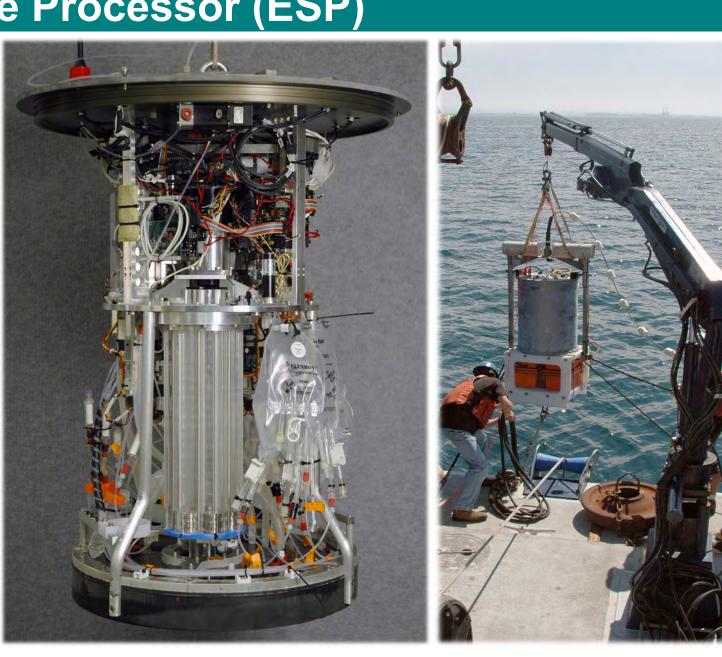


Coastal ecology and water quality have profound influences on socioeconomic well-being. Effective management of coastal environments requires clear understanding of the complex connections between human activities and the physical, chemical and dynamics of coastal biological ecosystems.

Informing the management process often requires unambiguous, quantitative knowledge of the abundance and activity of microorganisms that may negatively affect human health, recreation, fisheries, and tourism. The specificity and certainty with which microbial abundance and activity can be determined have been greatly augmented by molecular methods. Deployment of molecular methods on autonomous platforms is now bringing this valuable specificity and certainty into the realm of sustained, timely information flow to inform environmental management. The Environmental Sample Processor (ESP) is an autonomous robotic system that can acquire water samples, apply molecular methods to identify genes and gene products of target species, and report results to scientists and managers in near-real time. ESP is currently deployed on piers and moorings, excellent platforms for monitoring ecologically sensitive sites where human populations and activities may be concentrated. Fixed-location ESP deployments are supporting studies of potentially deleterious occurrences, including harmful algal blooms (HABs) and water quality degradation by anthropogenic constituents of land drainage. These phenomena may be coupled, thus a single ESP can run analyses relevant to understanding both. This poster highlights some recent studies employing ESP in the MBNMS and provides a look toward the near-term future of ESP mobilization on autonomous underwater vehicles (AUVs) and profiling drifters.

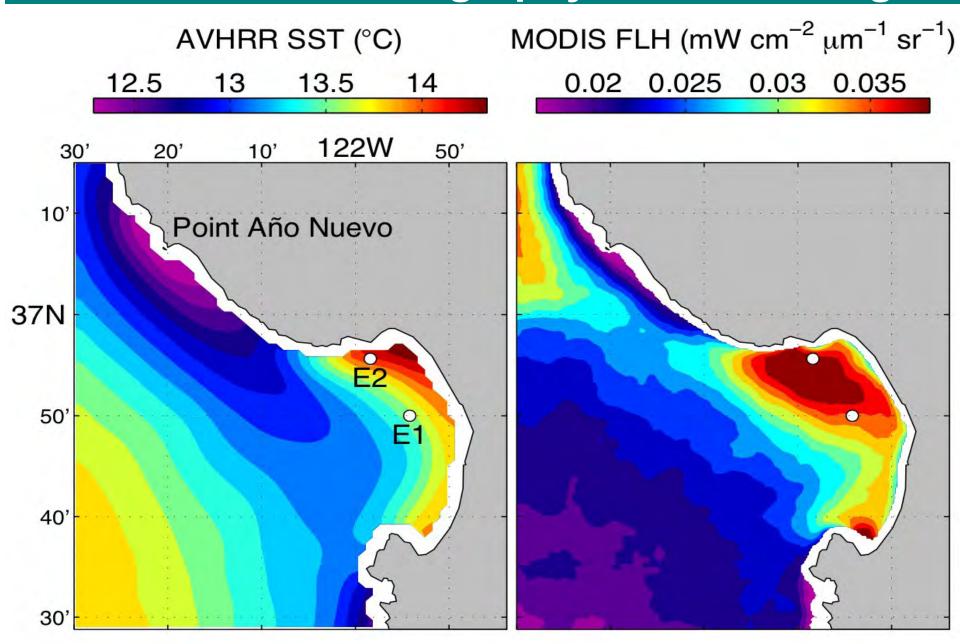
Environmental Sample Processor (ESP)

Application of molecular analytical techniques for identifying marine microbes, specific genes, and gene products has traditionally demanded collection of discrete samples, often in liter quantities, and transport of samples to a laboratory for processing. These requirements typically result in delays ranging from many hours to days between collection of material and its analysis. The Environmental Sample Processor (ESP) is a field-deployable electromechanical / fluidic system designed to autonomously collect water samples, concentrate microorganisms, and apply molecular probe technologies, providing information in near-real time (Scholin et al., 2009). We refer to this type of device as an ecogenomic sensor. Filterbased sandwich hybridization methodology enables direct detection of ribosomal RNA sequences diagnostic for groups of bacteria and archaea,



invertebrates, and harmful algal species. An antibody-based technique is used for detecting domoic acid (Doucette et al., 2009), an algal biotoxin. A four-channel quantitative polymerase chain reaction (qPCR) module is used for detection of a wide variety of microbial genes. The spectrum of microscopic marine life and life processes accessible to ESP is expanding with the development of new probes and molecular methods.

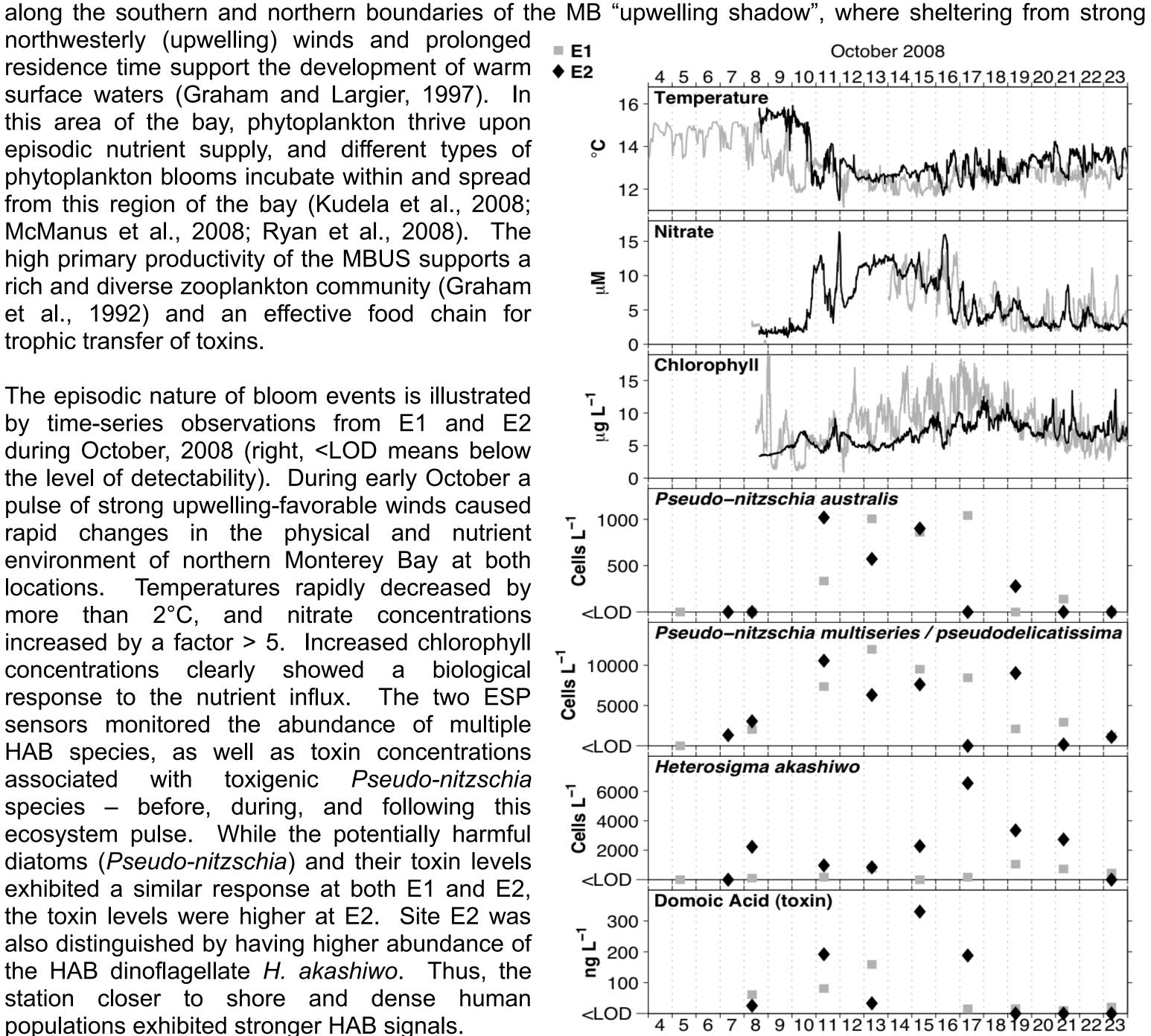
Oceanography of Harmful Algal Blooms (HABs)



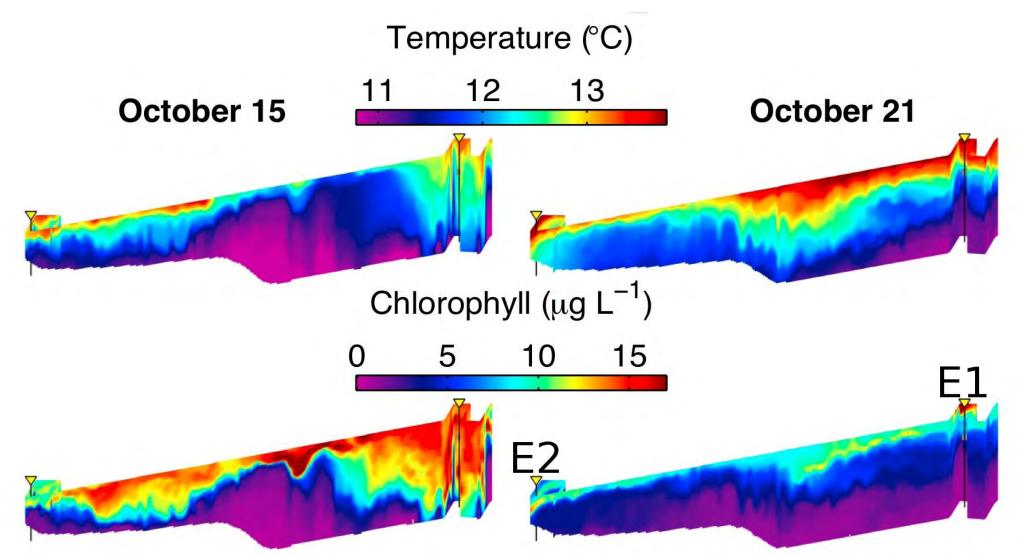
Understanding the oceanographic processes controlling the development of HABs is an essential step toward advancing prediction. The dynamic environment of Monterey Bay (MB) is an excellent natural laboratory in which to study HAB ecology. During intensive process studies in MB, we have deployed ESP mooring networks and integrated their molecular probe results with an array of environmental data from moorings, satellites and AUV surveys. The locations labeled E1 and E2 (left, 5-year climatology of satellite data) are where ESP moorings were deployed during month-long studies in 2007 and 2008. These locations are

northwesterly (upwelling) winds and prolonged residence time support the development of warm ϕ E2 surface waters (Graham and Largier, 1997). In this area of the bay, phytoplankton thrive upon episodic nutrient supply, and different types of phytoplankton blooms incubate within and spread from this region of the bay (Kudela et al., 2008; McManus et al., 2008; Ryan et al., 2008). The high primary productivity of the MBUS supports a rich and diverse zooplankton community (Graham et al., 1992) and an effective food chain for trophic transfer of toxins.

The episodic nature of bloom events is illustrated by time-series observations from E1 and E2 during October, 2008 (right, <LOD means below the level of detectability). During early October a pulse of strong upwelling-favorable winds caused rapid changes in the physical and nutrient environment of northern Monterey Bay at both locations. Temperatures rapidly decreased by more than 2°C, and nitrate concentrations increased by a factor > 5. Increased chlorophyll concentrations clearly showed a biological response to the nutrient influx. The two ESP sensors monitored the abundance of multiple HAB species, as well as toxin concentrations associated with toxigenic *Pseudo-nitzschia* species – before, during, and following this ecosystem pulse. While the potentially harmful diatoms (Pseudo-nitzschia) and their toxin levels exhibited a similar response at both E1 and E2, the toxin levels were higher at E2. Site E2 was also distinguished by having higher abundance of the HAB dinoflagellate *H. akashiwo*. Thus, the station closer to shore and dense human populations exhibited stronger HAB signals.



October 2008



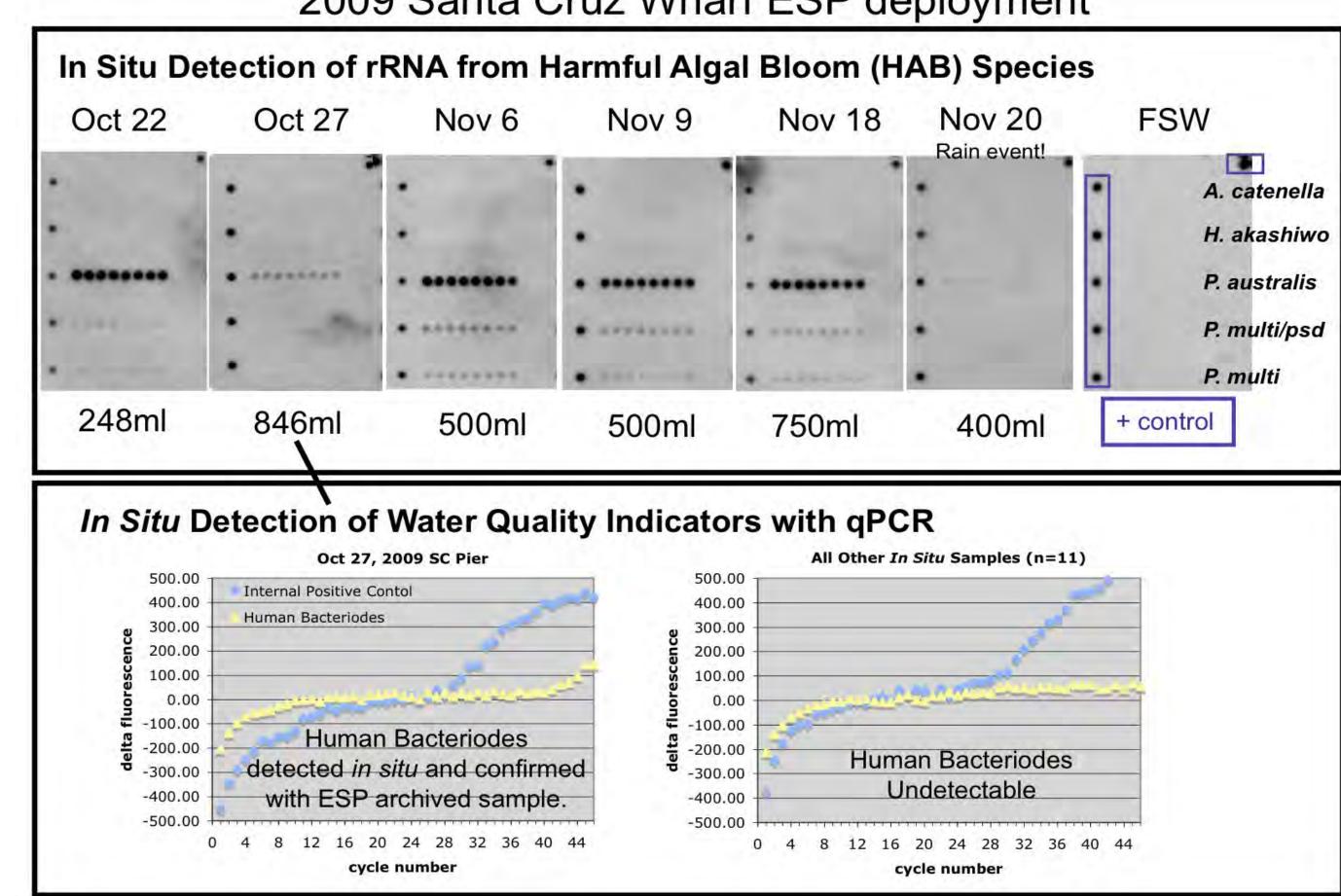
In concert with ESP network deployments, AUV surveys are regularly conducted to provide synoptic, multidisciplinary highresolution maps of the coastal environment in which microbiology is being studied. During the October 2008 ESP network deployment, changes in the northern bay water column around and between E1 and E2 were mapped by AUV surveys (left). This data provides valuable context on the oceanographic processes, patchiness and scales

of variability within which ESP sampling takes place. The two vertical sections shown here illustrate the warming and decrease in phytoplankton abundance at the end of the upwelling-generated bloom.

Simultaneous Monitoring of HABs and Water Quality Indicators

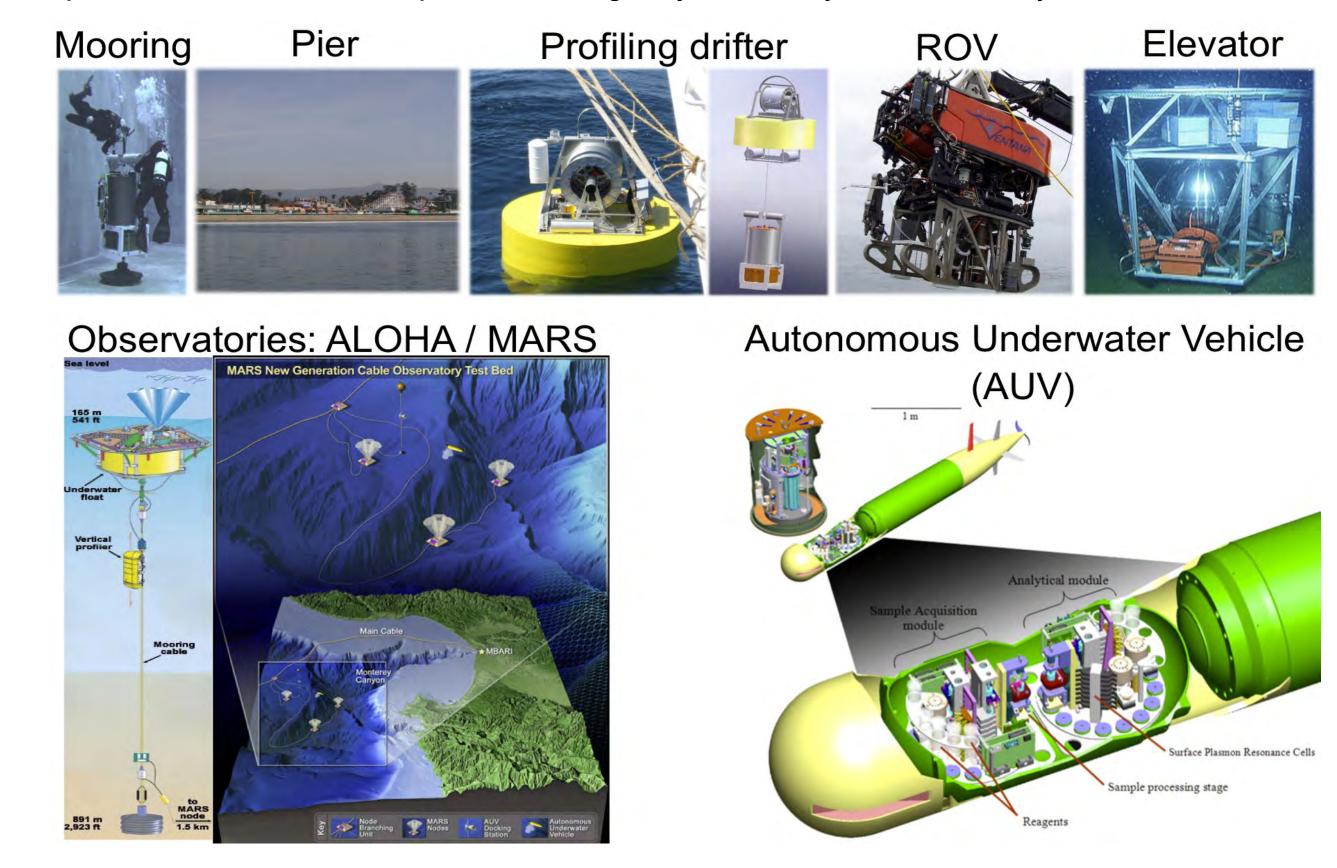
Nearshore water quality and human health can be impacted from the ocean-side by HABs and from the land-side by pathogens transported via coastal waterways. Further, the development of HABs in coastal waters can be linked to water quality degradation caused by constituents of land drainage. Because these coastal management issues may be linked and synergistically harmful, the ESP has been developed to simultaneously monitor HAB species and microorganisms associated with human sewage. During fall 2009, a time-series from the Santa Cruz Pier showed extremely high concentrations of HAB species that can cause amnesic shellfish poisoning. Quantitative PCR results also showed that bacteriodes from a human source were detected by ESP on October 27. These data are being integrated with UCSC monitoring data from Santa Cruz Pier.

2009 Santa Cruz Wharf ESP deployment



Platforms for Molecular Probing of Ocean Life

The ability to receive information from molecular probe techniques in near real-time creates opportunities to advance interdisciplinary ocean research. To explore these opportunities, the ESP has been developed for deployment on a variety of platforms, including moorings, piers, and benthic instrument nodes, from nearsurface to 4000 m depth. Fixed-location deployments are yielding unprecedented observations, yet many important questions in marine science require Lagrangian studies in which the processes and evolution of planktonic communities are tracked by following them and the environment in which they are transported. To advance Lagrangian studies, the current generation ESP (G2) is being deployed on a profiling drifter (below) in fall 2010. Miniaturization of ESP capabilities is in progress and will allow deployment of molecular probe techniques on autonomous underwater vehicles (AUVs). In parallel, the artificial intelligence of AUVs is advancing to permit autonomous feature detection, mapping and sampling, thus allowing onboard molecular probe resources to be expended intelligently, efficiently and effectively.



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